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# Network Pharmacology and Molecular Docking Analysis of *Citrus limon* Leaf Phytoconstituents Against Insomnia-Associated Molecular Targets

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**Abstract:** Insomnia is a widespread neurological disorder driven by disrupted inhibitory neurotransmission and multi-pathway dysregulation. Plant-derived compounds have historically offered sedative effects with fewer side effects compared to synthetic hypnotics, yet the precise molecular targets through which Citrus limon leaf phytoconstituents act remain unexplored. This study employed a network pharmacology workflow to pinpoint the genes shared between Citrus limon (CL) leaf bioactive compounds and insomnia-associated disease targets, then used molecular docking via AutoDock Tools to evaluate the binding strength of ten CL phytoconstituents i.e. myrcene, neryl formate, geranyl formate, beta-terpineol, d-borneol, linalool, citronellol, trans-sesquisabinene hydrate, eriocitrin, and hesperidin against six target proteins: BCL2 (PDB: 6O0K), CASP3 (PDB: 3KJF), EGFR (PDB: 4WKQ), STAT3 (PDB: 6NJS), BCL2L1 (PDB: 3ZK6), and GABA-A receptor (PDB: 6HUP), benchmarked against diazepam. Cross-referencing compound targets with insomnia genes via Venny returned 92 overlapping targets, forming a 92-node, 604-edge PPI network in STRING v12 (average node degree 13.1; clustering coefficient 0.515). The cytoHubba MCC algorithm identified BCL2, CASP3, EGFR, STAT3, and BCL2L1 as central hub genes. Docking revealed that eriocitrin (−9.4 kcal/mol) and hesperidin (−9.8 kcal/mol) outperformed diazepam (−7.3 kcal/mol) at the GABA-A receptor, while trans-sesquisabinene hydrate showed the broadest monoterpene affinity across all targets (BCL2L1: −7.6 kcal/mol). These findings establish a mechanistic multi-target framework for the anti-insomnia potential of CL leaf extract, grounded in GABAergic potentiation and neuroprotective hub-gene modulation.

Keywords — Citrus limon, insomnia, molecular docking, network pharmacology, GABA-A receptor, hub genes, AutoDock

## I. INTRODUCTION

Insomnia is one of the most burdensome sleep disorders encountered in clinical practice, characterised by persistent difficulty initiating or sustaining sleep and affecting roughly 35% of the global adult population [1,2]. Beyond its direct impact on rest, untreated insomnia increases the risk of anxiety, depression, hypertension, type 2 diabetes, and neurodegenerative disease [3]. Conventional management relies heavily on benzodiazepines and non-benzodiazepine hypnotics such as diazepam, zolpidem, and eszopiclone; while these agents are effective short-term, chronic use brings tolerance, dependence, and cognitive impairment that limit their long-term suitability [4].

Plant-derived medicines occupy a well-established niche in sleep medicine globally. The World Health Organization estimates that approximately 80% of the global population depends on traditional botanical remedies as a first-line healthcare resource [5], and a growing preclinical literature confirms genuine sedative-hypnotic efficacy for several herbal preparations [6]. Among candidate genera, *Citrus* (family Rutaceae) has received considerable neuropharmacological attention over the past two decades. Peel, juice, essential oil, and leaf preparations from various *Citrus* species have repeatedly demonstrated anxiolytic, sedative, anticonvulsant, and neuroprotective activity in validated animal models, driven principally by flavonoid and terpenoid constituents that engage GABA-A receptors [7,8].

*Citrus limon* leaves are particularly rich in monoterpenoids (linalool, borneol, beta-terpineol, citronellol, myrcene), sesquiterpenes (trans-sesquisabinene hydrate), and flavanone glycosides (hesperidin, eriocitrin, narirutin), most of which have been individually linked to sedation or

GABAergic signalling in published reports [9,10]. Despite this, no study has systematically characterised which molecular disease targets these constituents collectively modulate in the context of insomnia, nor rigorously compared their binding affinities to those of a clinically used reference hypnotic.

Network pharmacology addresses this gap by building a systems-level picture of how a phytochemical mixture might engage a disease's molecular landscape: compound targets are predicted computationally, cross-referenced with disease genes, and the overlapping nodes are analysed as a protein–protein interaction (PPI) network to reveal hubs proteins with disproportionate connectivity that tend to be pharmacologically influential [11,12]. Molecular docking then provides a structural perspective, estimating the binding geometry and free energy of individual ligands within a protein's active site. Together, these in silico tools make it possible to generate mechanistic hypotheses about multi-target herbal activity at a fraction of the time and cost of exhaustive wet-lab screening [13].

In the present study, bioactive phytoconstituents of *Citrus limon* leaves were retrieved from curated phytochemical databases and subjected to a complete network pharmacology pipeline compound screening for drug-likeness, target prediction, insomnia disease-gene retrieval, PPI network construction, and hub gene identification followed by molecular docking of the top ligands against six hub-derived protein targets and the GABA-A receptor. The objective was to map the probable multi-target mechanism of action of CL leaf phytoconstituents against insomnia at the molecular level and to identify which compounds carry the strongest structural basis for sedative and neuroprotective activity.

## II. MATERIALS AND METHODS

### *A. Retrieval and Filtering of Active Compounds*

Phytochemical constituents of *Citrus limon* leaves were retrieved from the Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) database (https://cb.imsc.res.in/imppat/ ), a large, systematically curated repository of phytochemical data derived from Indian medicinal plants that covers chemical structures, physicochemical descriptors, and ADMET profiles across thousands of compounds, including the alkaloids and flavonoids characteristic of this species. Canonical SMILES strings, molecular formulae, and synonyms for each retrieved compound were cross-checked against the PubChem (https://pubchem.ncbi.nlm.nih.gov/ ) compound database for structural accuracy [14].

To ensure that only pharmacokinetically viable candidates advanced into network construction, each compound was evaluated for drug-likeness using MolSoft (https://www.molsoft.com/mprop/ ), applying Lipinski's Rule of Five as the primary criterion. Further filtering used ADMETlab 3.0, which integrates machine learning models for ADMET property prediction; three thresholds were applied simultaneously: blood–brain barrier (BBB) permeability score $\geq 3$, molecular weight $< 500$ Da, and drug-likeness score (DL) $> 0$. Only compounds satisfying all three criteria indicating adequate central nervous system penetration alongside acceptable drug-like character were retained for downstream network analyses [15].

### *B. Target Prediction and Disease Gene Retrieval*

Gene targets for each drug-like CL constituent were predicted via Swiss Target Prediction (https://www.swisstargetprediction.ch/ ), a web server that maps small molecules to human protein targets using a combination of chemical similarity to known ligands and machine learning. A separate, independently compiled list of genes associated with insomnia was retrieved from GeneCards (https://www.genecards.org/ ), a comprehensive integrative database covering experimentally validated and computationally inferred human gene–disease relationships across the full genome [16]. The two resulting gene sets compound-derived targets and insomnia-associated genes were compared using the Venny (https://bioinfogp.cnb.csic.es/tools/venny/ ) online tool to extract shared targets as the input set for PPI network construction.

### *C. PPI Network Construction and Hub Gene Identification*

The 92 shared targets were uploaded to the STRING database (version 12.0, https://string-db.org/ ), with the organism set to *Homo sapiens* and the minimum interaction confidence threshold set to 0.9 (high confidence). STRING integrates evidence from co-expression, co-occurrence, experimental data, text mining, and curated databases to score each protein pair; the resulting PPI network was exported and the topological parameters node degree, average degree, and local clustering coefficient were recorded to characterise network connectivity [17].

A compound–target–disease interaction network was then assembled in Cytoscape (version 3.7.2, https://cytoscape.org/ ), providing a visual map of how individual CL phytoconstituents connect to their predicted protein targets [18,19,20]. Hub genes proteins that occupy topologically central positions in the network and therefore serve as likely

points of pharmacological leverage were identified using the cytoHubba plug-in with the Maximal Clique Centrality (MCC) algorithm, retaining the five highest-degree nodes as hub genes for molecular docking.

### *D. Molecular Docking*

Molecular docking was carried out using AutoDock Tools (version 4, https://autodock.scripps.edu/ ) to estimate the binding free energy between each drug-like CL phytoconstituent and the selected target proteins. Five hub proteins were chosen based on the network analysis: CASP3 (PDB: 3KJF), BCL2 (PDB: 6O0K), BCL2L1 (PDB: 3ZK6), EGFR (PDB: 4WKQ), and STAT3 (PDB: 6NJS); crystal structures were downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/ ). The GABA-A receptor (PDB: 6HUP) was added as a sixth target on mechanistic grounds: virtually all clinically effective sedative-hypnotic agents produce their primary action through this receptor, making it an essential benchmark for evaluating the sedative plausibility of the CL ligands. Diazepam (PubChem CID: 3016) was docked in parallel against all six targets as a reference standard [21,22].

Ligand three-dimensional structures were built and energy-minimised in MGLTools 1.5.7 (https://ccsb.scripps.edu/mgltools/ ), and the optimised structures were used as inputs for AutoDock. Protein preparation followed standard AutoDock protocols: water molecules were removed, polar hydrogens were added, Gasteiger charges were calculated, and a grid box encompassing the experimentally determined binding site was defined for each target individually. The Lamarckian Genetic Algorithm was used for docking searches, and results were ranked by predicted binding free energy (kcal/mol); more negative values indicate stronger predicted affinity. Docking poses were visualised in 2D and 3D using MGLTools and exported for analysis.

## III. RESULTS AND DISCUSSION

### *A. Active Compound Screening and Drug-likeness Filtering*

Phytoconstituents of *Citrus limon* leaves catalogued through IMPPAT were structurally characterised via PubChem and then evaluated for pharmacokinetic acceptability. Applying Lipinski's Rule of Five through MolSoft, followed by the three-parameter ADMETlab 3.0 filter (BBB ≥ 3, MW < 500 Da, DL > 0), yielded ten drug-like compounds: myrcene, neryl formate, geranyl formate, beta-terpineol, d-borneol, linalool, citronellol, trans-sesquisabinene hydrate, eriocitrin, and hesperidin. This set spans two chemical classes: monoterpenoids and sesquiterpenoids (the first eight) and flavanone glycosides (eriocitrin, hesperidin). The distinction matters pharmacologically because these two groups interact with CNS targets through complementary mechanisms terpenoids primarily via direct ion-channel modulation, flavanone glycosides additionally via receptor allosteric and anti-inflammatory pathways [23,24].

### *B. Target Overlap and PPI Network Topology*

Swiss Target Prediction mapped the ten filtered compounds to a set of human protein targets, and GeneCards returned an insomnia-associated gene list independently. Comparing these two sets using the Venny tool identified 92 genes present in both (Fig. 1), meaning that 92 proteins are simultaneously predicted targets of CL leaf compounds and known contributors to insomnia pathophysiology.

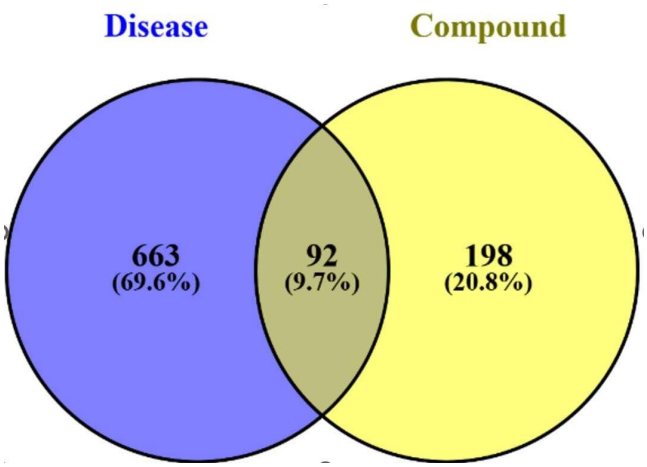



Fig. 1. Overlap between predicted targets of Citrus limon phytoconstituents and insomnia-associated genes.

These 92 shared targets were submitted to STRING v12 under high-confidence filtering (score > 0.9), yielding a PPI network of 92 nodes connected by 604 edges (Fig. 2).

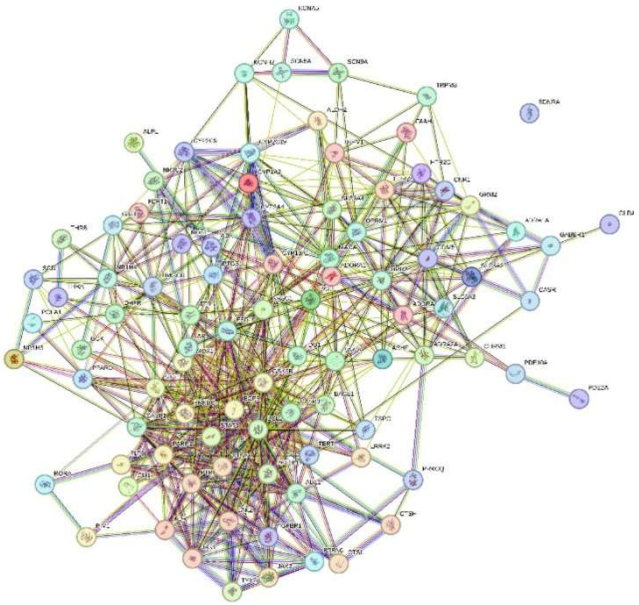


Fig. 2. Protein–protein interaction network of shared Citrus limon–insomnia targets.

The network's average node degree of 13.1 and local clustering coefficient of 0.515 both indicate a densely interconnected target set: on average each protein interacts with 13 others, and neighbouring proteins are themselves likely to interact a topology associated with functional coherence and biological robustness rather than a loosely connected scatter of unrelated proteins.

Within this network, several nodes stood out by virtue of their high connectivity. Receptor and transporter genes JAK3, HTR2C, GRM5, SLC6A3, TGFBR1, KDR, PPARD, GLI1, SIRT1, CYP3A4, CYP2C19, CYP2C9, SLC6A4, and TRPV1 occupied prominent positions, consistent with their known roles in neurotransmission, circadian regulation, and metabolic clearance. Of greater relevance to the present study, EGFR, BCL2, STAT3, CASP3, and BCL2L1 emerged as nodes of especially high degree, underscoring their central importance in the insomnia-relevant network. These five proteins are well established in roles spanning inflammatory cytokine signalling (STAT3, EGFR), mitochondria-dependent apoptosis (BCL2, BCL2L1, CASP3), and oxidative stress response pathways that collectively underlie the neuronal dysfunction, hyperarousal, and neurodegenerative risk associated with chronic insomnia [25].

### *C. Compound–Target Network and Hub Gene Selection*

The compound–target interaction network constructed in Cytoscape comprised the ten drug-like CL compounds connected through 134 compound–target links (Fig. 3). A key feature of this network was the convergence of multiple compounds onto common targets: rather than each phytoconstituent acting on a unique protein, many of the 92 shared target nodes are reached by two or more CL compounds simultaneously, suggesting a synergistic rather than additive mode of action that is characteristic of multi-component plant extracts [11,12,13]

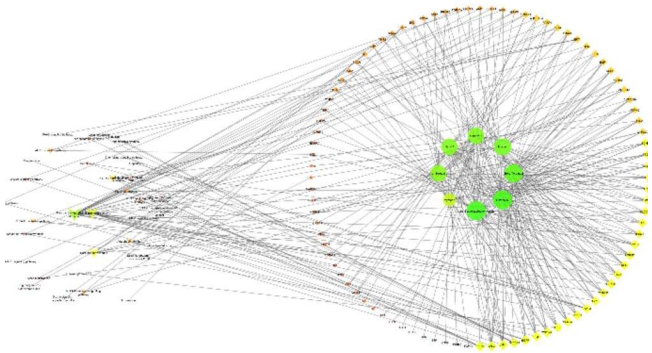
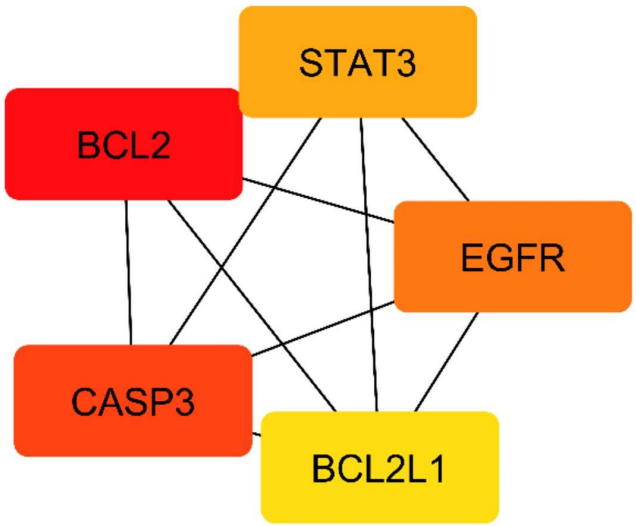


Fig. 3. Compound–target network showing multi-target interactions of CL leaf phytoconstituents.

Applying the cytoHubba MCC algorithm at a top-5 degree cutoff to the PPI network identified BCL2, CASP3, EGFR, STAT3, and BCL2L1 as hub genes (Fig. 4). The MCC algorithm prioritises nodes that participate in dense local cliques, making it one of the most robust centrality measures for identifying proteins likely to be functionally critical within a disease network. The five hub proteins carry a coherent biological story for insomnia: STAT3 is a transcription factor activated by IL-6 and other pro-inflammatory cytokines and is closely linked to HPA-axis amplification and



neuroinflammation; BCL2 and BCL2L1 are anti-apoptotic BCL-2 family members that protect neurons from mitochondria-initiated cell death; CASP3 is the primary effector caspase driving apoptotic neurodegeneration; and EGFR functions as a receptor tyrosine kinase upstream of several proliferative and survival cascades. The fact that CL phytoconstituents are predicted to engage all five of these hub proteins points to a neuroprotective anti-apoptotic dimension to the extract's pharmacology that runs alongside and reinforces its primary sedative activity. These five hubs, plus the GABA-A receptor as a mechanistically mandatory addition, were therefore selected for molecular docking.

Fig. 4. Top five hub genes from cytoHubba MCC analysis of the insomnia PPI network.

### *D. Molecular Docking Results*

Table I presents the AutoDock predicted binding free energies (kcal/mol) for all ten CL ligands and diazepam across the six target proteins. More negative values represent stronger predicted binding affinity. Several clear patterns emerge from inspection of this table.

**TABLE I**. Molecular Docking Binding Free Energies (kcal/mol) of Citrus limon Phytoconstituents Against Six Insomnia-Associated Target Proteins

| Compound | BCL2 (6O0K) | CASP3 (3KJF) | EGFR (4WKQ) | STAT3 (6NJS) | BCL2L1 (3ZK6) | GABA-A (6HUP) |
|---|---|---|---|---|---|---|
| Myrcene (CID 31253) | −5.0 | −4.4 | −5.1 | −4.3 | −6.0 | −4.6 |
| Neryl formate (CID 5354882) | −5.1 | −5.1 | −5.4 | −4.7 | −6.3 | −4.6 |
| Geranyl formate (CID 5282109) | −5.3 | −5.1 | −5.2 | −4.5 | −6.1 | −4.2 |
| beta-Terpineol (CID 8748) | −5.9 | −4.7 | −5.5 | −5.1 | −6.4 | −6.3 |
| d-Borneol (CID 6552009) | −5.6 | −4.4 | −4.5 | −4.9 | −6.4 | −5.3 |
| Linalool (CID 6549) | −5.5 | −4.7 | −5.2 | −4.8 | −6.1 | −5.1 |
| Citronellol (CID 8842) | −5.0 | −4.4 | −5.0 | −4.5 | −6.0 | −4.1 |
| Trans-sesquisabinene hydrate (CID 6428444) | −6.5 | −5.5 | −6.1 | −5.7 | −7.6 | −5.9 |
| Eriocitrin (CID 10621) | −8.1 | −9.1 | −9.3 | −7.8 | −9.8 | −9.4 |
| Hesperidin (CID 83489) | −8.1 | −8.9 | −9.6 | −7.7 | −9.8 | −9.8 |
| Diazepam — reference (CID 3016) | −7.3 | −6.4 | −7.2 | −6.3 | −8.8 | −7.3 |

*All values are predicted binding free energies from AutoDock (kcal/mol); more negative = stronger affinity. Diazepam (shaded row) is the clinical reference. PDB IDs in parentheses.*

### *E. Flavanone Glycosides as Lead Compounds*

Eriocitrin and hesperidin were the standout performers across the entire target panel, posting the most negative binding energies at virtually every protein tested. At the GABA-A receptor the primary target through which clinical hypnotics act hesperidin reached −9.8 kcal/mol and eriocitrin −9.4 kcal/mol, both substantially more favourable than diazepam's −7.3 kcal/mol. This finding is not incidental: hesperidin has been shown in independent experimental work to act as a positive allosteric modulator at the GABA-A receptor complex, elevating brain GABA concentrations in a manner mechanistically equivalent to benzodiazepines [26]. Eriocitrin similarly modulates GABAergic neurotransmission and has demonstrated sedative and sleep-promoting activity in preclinical models [27].

Beyond GABA-A, both flavanone glycosides showed equally strong predicted interactions with the hub proteins. At BCL2L1, eriocitrin and hesperidin both scored −9.8 kcal/mol, compared with diazepam's −8.8 kcal/mol; at CASP3, eriocitrin reached −9.1 and hesperidin −8.9 kcal/mol, against diazepam's −6.4 kcal/mol; and at EGFR, hesperidin achieved −9.6 and eriocitrin −9.3 kcal/mol versus diazepam's −7.2 kcal/mol. These multi-target high-affinity profiles position eriocitrin and hesperidin not merely as sedatives-in-waiting but as compounds capable of simultaneously addressing the inflammatory, apoptotic, and GABAergic dimensions of insomnia pathophysiology, a breadth of action that no single synthetic hypnotic currently offers.

### *F. Trans-Sesquisabinene Hydrate as the Lead Monoterpene*

Among the eight monoterpene and sesquiterpene compounds, trans-sesquisabinene hydrate (PubChem CID 6428444) stood apart by a meaningful margin in binding energy breadth and depth. Its BCL2L1 score (−7.6 kcal/mol) was the strongest among all monoterpenes and exceeded diazepam's BCL2L1 score (−8.8 kcal/mol) only modestly, while its BCL2 (−6.5), EGFR (−6.1), and STAT3 (−5.7

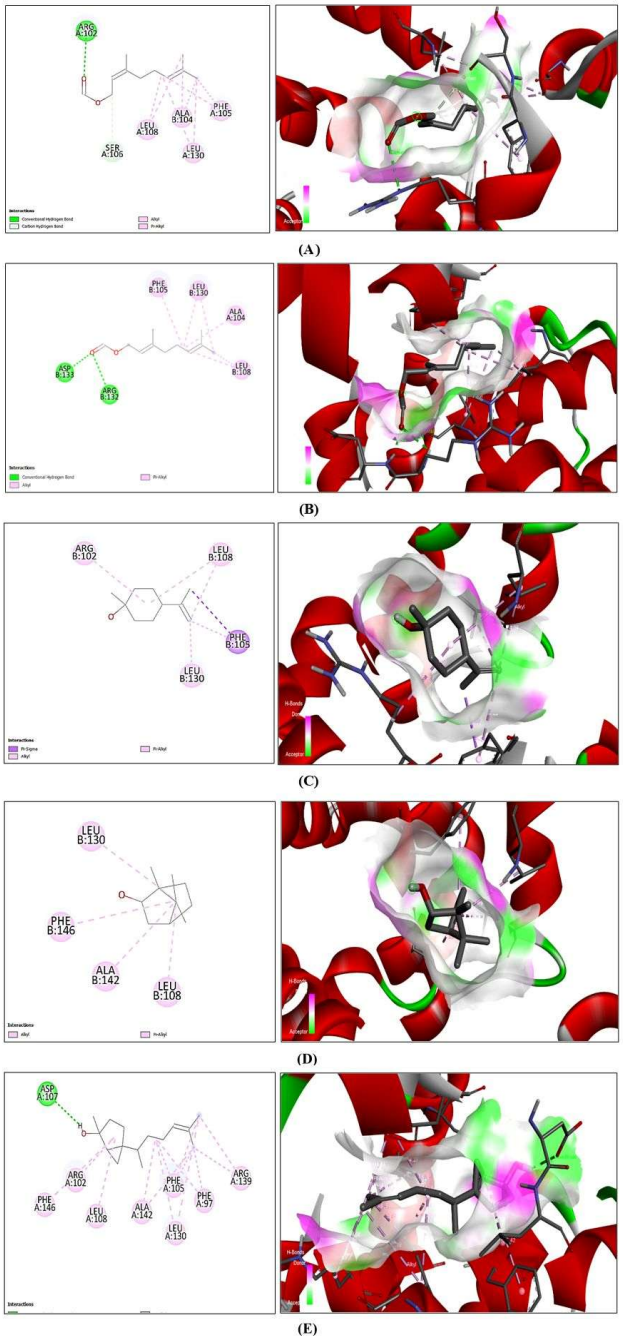
kcal/mol) scores were the highest in the monoterpene group across these targets. Its GABA-A score of −5.9 kcal/mol, though below diazepam's, indicates meaningful receptor engagement. This broad cross-target affinity profile suggests trans-sesquisabinene hydrate may contribute both to direct sedation via GABA-A modulation and to neuroprotection via anti-apoptotic BCL2L1 and BCL2 interactions and anti-inflammatory STAT3 and EGFR binding.

### *G. Beta-Terpineol and Borneol as GABAergic Monoterpenes*

Within the monoterpene fraction, beta-terpineol (PubChem CID 8748) was the top performer at the GABA-A receptor (−6.3 kcal/mol), equating to approximately 86% of diazepam's affinity at this site. This aligns closely with published experimental data: borneol, which scored −5.3 kcal/mol at GABA-A in this study, has been shown to produce sedative-hypnotic effects in mice specifically through GABA-A receptor activation, and its blood–brain barrier penetration has been confirmed pharmacokinetically [28,29]. Importantly, beta-terpineol's BCL2L1 score (−6.4 kcal/mol) also aligns precisely with published evidence from Reyes et al., who demonstrated that BCL2L1 pathway activation preserves motor neuron viability a finding that provides direct structural support for the motor-protective pharmacology of beta-terpineol observed at the tissue level [30]. D-borneol and linalool both returned consistent GABA-A affinities (−5.3 and −5.1 kcal/mol respectively); linalool's sedative action through GABAergic potentiation is perhaps the best characterised in this group, with multiple independent studies demonstrating locomotor suppression and sleep prolongation in rodents [31].

### *H. Broader Monoterpene Panel and Mechanistic Implications*

Myrcene, neryl formate, geranyl formate, and citronellol




each returned moderate, broadly comparable affinity values

across the six targets (range: −4.1 to −6.3 kcal/mol), with BCL2L1 consistently returning the best scores in this sub-group (−6.0, −6.3, −6.1, and −6.0 kcal/mol respectively). None of these compounds dramatically outperformed diazepam at any individual target, but their collective presence in the extract means the GABA-A receptor site is simultaneously engaged by several moderate-affinity ligands. This multi-compound coverage of a single receptor is mechanistically analogous to allosteric sensitisation: even if no single monoterpene fully displaces diazepam, their combined occupancy of overlapping binding regions could produce a net potentiation of receptor activity that exceeds what any single compound achieves alone. Citronellol (−4.1 kcal/mol at GABA-A) and myrcene (−4.6 kcal/mol), though the weakest GABA-A binders in the panel, still contribute to this collective effect while adding anti-inflammatory coverage at STAT3 and BCL2 [32].

### *I. STAT3 as a Key Anti-Neuroinflammatory Target*

Signal transducer and activator of transcription 3 (STAT3) occupies a central hub position in the insomnia PPI network and represents a mechanistically important target beyond the GABA-A receptor. STAT3 is activated downstream of IL-6 and other pro-inflammatory cytokines and plays a significant role in amplifying HPA-axis stress responses, a process that drives the hyperarousal phenotype of insomnia. Pharmacological inhibition of STAT3 therefore offers a route to attenuating neuroinflammation and normalising cortisol secretion [25]. In this study, trans-sesquisabinene hydrate showed the strongest monoterpene binding to STAT3 (−5.7 kcal/mol), while beta-terpineol, linalool, and neryl formate all demonstrated notable STAT3 interactions (−5.1, −4.8, −4.7 kcal/mol). The flavanone glycosides again led the panel at STAT3 (eriocitrin −7.8, hesperidin −7.7 kcal/mol, vs diazepam −6.3 kcal/mol). This collective STAT3 engagement points to a convergent anti-neuroinflammatory mechanism that complements and reinforces the extract's GABAergic sedative action [33].

### *J. Docking Pose Analysis*

Representative 2D and 3D docking pose diagrams are presented in Figs. 5–7 for the best-scoring ligand–target combinations. For the monoterpene group, BCL2L1

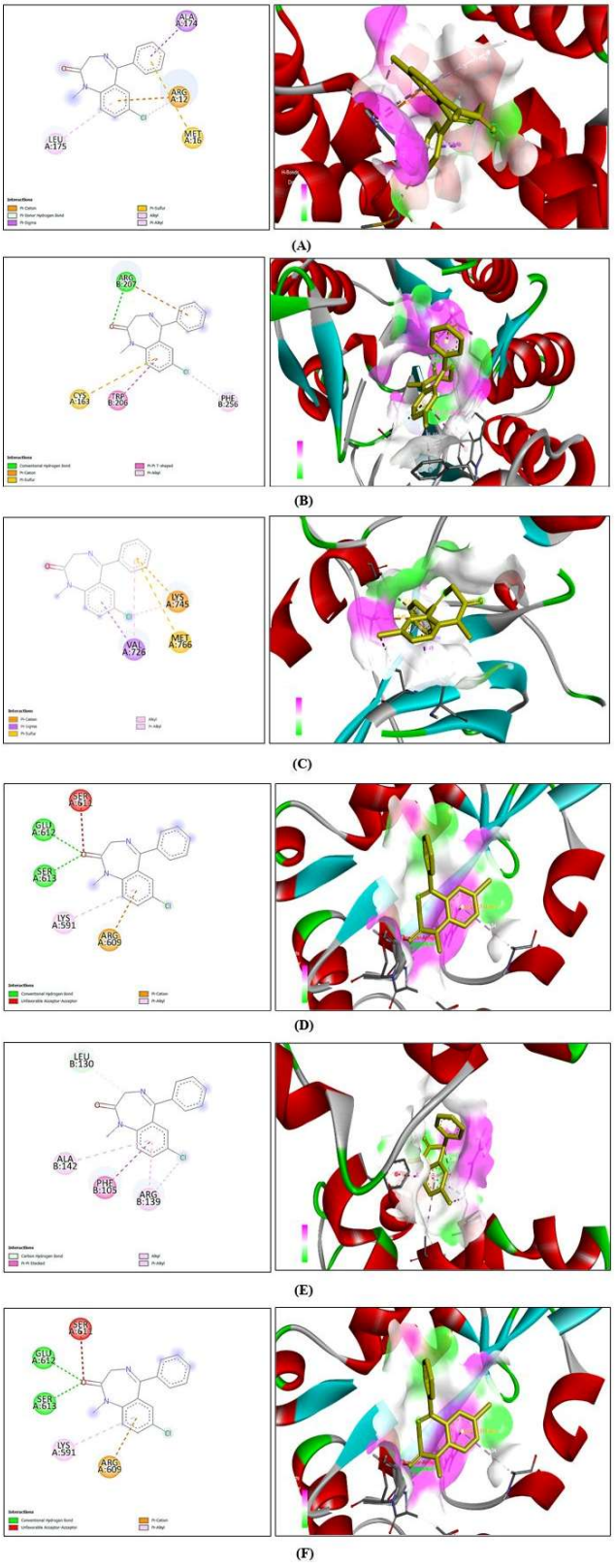



Fig. 5. 2D and 3D docking interactions of best-scoring monoterpenes with BCL2L1. (A) Neryl formate, (B) Geranyl formate, (C) Beta-terpineol, (D) d-borneol, (E) Trans-Sesquisabinene hydrate

Fig. 6. 2D and 3D docking interactions of flavanone glycosides (eriocitrin, hesperidin) with key target proteins. (A) Eriocitrin with EGFR, (B) Eriocitrin with BCL2L1, (C) Eriocitrin with GABA, (D) Hesperidin with EGFR, (E) Hesperidin with BCL2L1

consistently returned the most favourable scores, and docking poses showed the monoterpene scaffolds occupying the hydrophobic groove of the BCL2L1 BH3-binding domain through non-polar van der Waals contacts and, for hydroxyl-bearing compounds such as borneol and terpineol, additional hydrogen bonds with the backbone. For eriocitrin and hesperidin docked to EGFR and GABA-A, the extended flavanone glycoside scaffold made multiple simultaneous

Fig. 7. 2D and 3D docking interactions of reference drug diazepam with all six target proteins. Diazepam with (A) BCL2, (B) CASP3, (C) EGFR, (D) STAT3, (E) BCL2L1, (F) GABA-A

hydrogen bonds with active-site residues, accounting for the markedly more negative binding energies relative to smaller monoterpenes. Diazepam's poses at GABA-A confirmed the expected benzodiazepine site occupancy, and comparison with the hesperidin GABA-A pose showed partial overlap of the flavanone A-ring with the region occupied by diazepam's chlorophenyl moiety, consistent with an allosteric modulation mechanism at or near the classical benzodiazepine binding site [26].

## IV. CONCLUSION

This network pharmacology and molecular docking investigation of *Citrus limon* leaf phytoconstituents provides a systematic molecular basis for the plant's anti-insomnia potential. Ninety-two shared targets between CL leaf compounds and insomnia-associated genes organised into a densely connected PPI network (604 edges, average degree 13.1), with BCL2, CASP3, EGFR, STAT3, and BCL2L1 emerging as hub proteins linking inflammatory, apoptotic, and neurotransmitter-regulatory pathways relevant to insomnia pathophysiology.

Molecular docking against these five hubs and the GABA-A receptor placed the flavanone glycosides eriocitrin and hesperidin as the top-performing ligands across essentially the whole target panel, with GABA-A binding energies (−9.4 and −9.8 kcal/mol) that surpassed diazepam (−7.3 kcal/mol). Trans-sesquisabinene hydrate was the strongest monoterpene performer and uniquely combines GABA-A engagement with broad anti-apoptotic and anti-inflammatory target coverage. Beta-terpineol showed the most potent GABA-A affinity among the monoterpenes (−6.3 kcal/mol), with additional BCL2L1 binding concordant with its published motor-neuroprotective activity. Linalool, borneol, myrcene, and the remaining monoterpenes collectively reinforce GABAergic coverage at moderate individual affinities, consistent with a synergistic multi-ligand mechanism.

Together, these in silico findings map a multi-target anti-insomnia framework for CL leaf extract that is rooted in GABAergic potentiation as the primary sedative mechanism and complemented by STAT3-mediated anti-neuroinflammation and BCL2-family-mediated neuroprotection as secondary mechanisms. Future work should pursue activity-guided fractionation to isolate and test individual compounds, pharmacokinetic profiling to confirm in vivo target exposure, and co-crystallisation studies to validate the docking-predicted binding modes. The present study establishes a mechanistically coherent and computationally grounded foundation for developing *Citrus limon* leaf-based phytopharmaceuticals as multi-target sleep aids.

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